METHOD 200.3

SAMPLE PREPARATION PROCEDURE FOR SPECTROCHEMICAL DETERMINATION OF TOTAL RECOVERABLE ELEMENTS IN BIOLOGICAL TISSUES

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1. SCOPE AND APPLICATION

1.1 This method provides sample preparation procedures for the determination of total recoverable elements in biological tissue samples.

1.2 This method is applicable to the following elements:

Analyte		Chemical Abstract Services Registry Numbers (CASRN)
Aluminum	(Al)	7429-90-5
Antimony	(Sb)	7440-36-0
Arsenic	(As)	7440-38-2
Barium	(Ba)	7440-39-3
Beryllium	(Be)	7440-41-7
Cadmium	(Cd)	7440-43-9
Calcium	(Ca)	7440-70-2
Chromium	(Cr)	7440-47-3
Cobalt	(Co)	7440-48-4
Copper	(Cu)	7440-50-8
Iron	(Fe)	7439-89-6
Lead	(Pb)	7439-92-1
Lithium	(Li)	7439-93-2
Magnesium	(Mg)	7439-95-4
Manganese	(Mn)	7439-96-5
Mercury	(HG)	7439-97-6
Molybdenum	(Mo)	7439-98-7
Nickel	(Ni)	7440-02-0
Phosphorus	(P)	7723-14-0
Potassium	(K)	7440-09-7
Selenium	(Se)	7782-49-2
Silver	(Ag)	7440-22-4
Sodium	(Na)	7440-23-5
Strontium	(Sr)	7440-24-6
Thallium	(Tl)	7440-28-0
Thorium	(Th)	7440-29-1
Uranium	(U)	7440-61-1
Vanadium	(V)	7440-62-2
Zinc	(Zn)	7440-66-6

1.3 Samples prepared by this method can be analyzed by inductively coupled plasma-atomic emission spectrometry (ICP-AES) Method 200.7, "Determination of Metals and Trace Elements by Inductively Coupled Plasma-Atomic Emission Spectrometry," inductively coupled plasma-mass spectrometry (ICP-MS) Method 200.8, "Determination of Metals

and Trace Elements by Inductively Coupled Plasma-Mass Spectrometry," and stabilized temperature platform graphite furnace atomic absorption (STGFAA), Method 200.9, "Determination of Trace Elements by Stabilized Temperature Graphite Furnace Atomic Absorption Spectrometry". See analytical methods mentioned for selection of the appropriate method for determination of a specific analyte.

2. SUMMARY OF METHOD

2.1 Up to 5 g of a frozen tissue sample is transferred to a 125 mL flask. The tissue is digested with nitric acid, hydrogen peroxide and heat. This digestion results in a clear solution that is then analyzed by mass or atomic spectrometry methods. The determined metal concentration is reported in microgram/gram (µg/g) wet tissue weight.

3. DEFINITIONS

3.1 TOTAL RECOVERABLE - The concentration of analyte determined to be in either a solid sample or an unfiltered aqueous sample following treatment by refluxing with hot dilute mineral acid.

3.2 LABORATORY REAGENT BLANK (LRB) - A solution of reagents that is treated exactly as a sample including exposure to all glassware and equipment that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, reagents, or apparatus.

4. INTERFERENCES

4.1 Chromium contamination of biological samples from the use of stainless steel has been reported.[4] Use of special cutting implements and dissecting board made from materials that are not of interest is recommended. Knife blades made of titanium with Teflon handles have been successfully used.

4.2 In sample preparation, contamination is of prime concern. The work area, including bench top and fume hood, should be periodically cleaned in order to eliminate environmental contamination.

4.3 Chemical interferences are matrix dependent and cannot be predicted.

5. SAFETY

5.1 All personnel handling environmental samples known to contain or to have been in contact with human waste should be immunized against known disease causative agents.

5.2 Material safety data sheets for all chemical reagents should be available to and understood by all personnel using this method. Concentrated nitric and hydrochloric acids are moderately toxic and extremely irritating to skin and mucus membranes. Hydrogen peroxide

is a strong oxidizing reagent. Use these reagents in a hood whenever possible and if eye or skin contact occurs, flush with large volumes of water. Always wear safety glasses or a shield for eye protection when working with these reagents.

6. APPARATUS AND EQUIPMENT

6.1 LABWARE - For determination of trace levels of elements, contamination and loss are of prime consideration. Potential contamination sources include improperly cleaned laboratory apparatus and general contamination within the laboratory environment from dust, etc. A clean laboratory work area designated for trace element sample handling must be used. Sample containers can introduce positive and negative errors in the determination of trace elements by contributing contaminants through surface desorption/leaching, or depleting element concentrations through adsorption processes. All reusable labware (glass, quartz, polyethylene, Teflon, etc.), including the sample container, should be cleaned prior to use or shown to be contaminant free. Labware should be soaked overnight and thoroughly washed with laboratory-grade detergent and water, rinsed with water, and soaked for four hours in a mixture of dilute nitric and hydrochloric acid (1+2+9), followed by rinsing with water, ASTM type I water and oven drying.

NOTE: Chromic acid must not be used for cleaning glassware.

6.1.1 Glassware - Volumetric flasks, graduated cylinders and 125-mL Erlenmeyer flasks.

6.1.2 Assorted calibrated pipettes.

6.1.3 Wash bottle - One piece stem, Teflon FEP bottle with Tefzel ETFE screw closure, 125-mL capacity.

6.2 SAMPLE PROCESSING EQUIPMENT

6.2.1 Balance - Analytical, capable of accurately weighing to 0.1 mg.

6.2.2 Hot Plate - (Corning PC100 or equivalent). An oscillating hot plate will aid in sample digestion.

6.3 TISSUE DISSECTING EQUIPMENT

6.3.1. Dissecting Board: Polyethylene or other inert, nonmetallic material, any non-wetting, easy-to-clean or disposable surface is suitable. Adhesive backed Teflon or plastic film may be convenient to use.

6.3.2 Forceps: Plastic, Teflon or Teflon coated.

6.3.3 Surgical Blades: Disposable stainless steel with stainless steel or plastic handle (Sect. 4.1).

6.3.4 Scissors: Stainless steel.

6.3.5 Plastic bags with watertight seal, metal free.

6.3.6 Label tape: Self-adhesive, vinyl coated marking tape, solvent resistant, usable for temperatures from +121°C to -23°C.

6.3.7 Polyvinyl chloride or rubber gloves, talc-free.

7. REAGENTS AND CONSUMABLE MATERIALS

7.1 Reagents may contain elemental impurities which might affect analytical data. High-purity reagents should be used whenever possible. All acids used for this method must be of ultra high-purity grade.

7.1.1 Nitric acid, concentrated (sp.gr. 1.41).

7.1.2 Hydrochloric acid, concentrated (sp.gr. 1.19).

7.1.3 Hydrogen peroxide (30%)

7.2 WATER - For all sample preparation and dilutions, ASTM type I water (ASTM D1193) is required. Suitable water may be prepared by passing distilled water through a mixed bed of anion and cation exchange resins.

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Appropriate individual tissue samples should be taken soon after collection and must be taken prior to freezing[2]. If dissection of the tissue cannot be performed immediately after collection, it should be placed in a plastic bag (Sect. 6.3.5), sealed and placed on ice or refrigerated at approximately 4°C.

8.2 Prior to dissection, the tissue should be rinsed with metal-free water and blotted dry. Dissection should be performed within 24 hours of collection. Each individual tissue sample should also be rinsed with metal-free water, blotted dry, and frozen at ≤-20°C (dry ice).

8.3 Tissue samples of up to 5 g should be taken using a special implement (Sect. 4.1) and handled with plastic forceps (Sect. 6.3.2)[3,4].

8.4 A maximum holding time for frozen samples has not been determined.

9. CALIBRATION AND STANDARDIZATION

9.1 Not applicable. Follow instructions given in the analytical method selected.

10. QUALITY CONTROL

10.1 Each laboratory determining total recoverable elements is required to operate a formal quality control (QC) program. The minimum requirements of a QC program consist of an initial demonstration of laboratory capability and analysis of laboratory reagent blanks and fortified blanks and samples as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of data generated.

10.2 Specific instructions on accomplishing the described aspects of the QC program are discussed in the analytical methods.

11. PROCEDURE

11.1 Sample Preparation - Place up to a 5 g subsample of frozen tissue into a 125-mL erlenmeyer flask. Any sample spiking solutions should be added at this time and allowed to be in contact with the sample prior to addition of acid.

11.2 Add 10 mL of concentrated nitric acid and warm on a hot plate until the tissue is solubilized. Gentle swirling the samples or use of an oscillating hot plate will aid in this process.

11.3 Increase temperature to near boiling until the solution begins to turn brown. Cool sample, add an additional 5 mL of concentrated nitric acid and return to the hot plate until the solution once again begins to turn brown.

11.4 Cool sample, add an additional 2 mL of concentrated nitric acid, return to the hot plate and reduce the volume to 5-10 mL. Cool sample, add 2 mL of 30% hydrogen peroxide, return sample to the hot plate and reduce the volume to 5-10 mL.

11.5 Repeat Sect. 11.4 until the solution is clear or until a total of 10 mL of peroxide has been added. **NOTE:** A laboratory reagent blank is especially critical in this procedure because the procedure concentrates any reagent contaminants.

11.6 Cool the sample, add 2 mL of concentrated hydrochloric acid, return to the hot plate and reduce the volume to 5 mL.

11.7 Allow the sample to cool and quantitatively transfer to a 100-mL volumetric flask. Dilute to volume with ASTM type I water, mix, and allow any insoluble material to separate. The sample is now ready for analysis by either ICP-AES or STGFAA. For analysis by ICP-MS an additional dilution (1+4) is required.

11.8 Sample Analysis - Use one of the analytical methods listed in Sect. 1.3.

12. CALCULATIONS

12.1 Not applicable. Discussed in analytical methods listed in Sect. 1.3.

13. PRECISION AND ACCURACY

13.1 Not applicable. Available data included in analytical methods listed in Sect. 1.3.

14. REFERENCES

1. Versieck, J., and F. Barbier, "Sample Contamination as A Source of Error in Trace-Element Analysis of Biological Samples," Talanta, Vol. 29, pp. 973-984, 1982.

2. Ney, J. J., and M. G. Martin, "Influences of Prefreezing on Heavy Metal Concentrations in Bluegill Sunfish," Water Res., Vol. 19, No. 7, pp. 905-907, 1985.

3. "The Pilot National Environmental Specimen Bank," NBS Special Publication 656, U. S. Department of Commerce, August, 1983.

4. Koirtyohann, S. R., and H. C. Hopps, "Sample Selection, Collection, Preservation and Storage for Data Bank on Trace Elements in Human Tissue," Federation Proceedings, Vol. 40, No. 8, June, 1981.